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Abstract

Objective

We sought to characterise *C9orf72* expansions in relation to genetic ancestry and age at onset (AAO), and to use these parameters to discriminate the behavioural from the language variant syndrome, in a large pan-European cohort of frontotemporal lobar degeneration (FTLD) cases.

Methods

We evaluated expansions frequency in the entire cohort (n=1396; bvFTD [n=800], PPA [n=495] and FTLD-MND [n=101]). We then focused on the bvFTD and PPA cases and tested for association between expansion status, syndromes, genetic ancestry, and AAO applying statistical tests comprising Fisher's Exact, ANOVA with Tukey *post-hoc* tests, and logistic and non-linear mixed-effects model regressions.

Results

We found *C9orf72* pathogenic expansions in 4% of all cases (56/1396). Expansion carriers differently distributed across syndromes: 12/101 FTLD-MNDs (11.9%), 40/800 bvFTDs (5%) and 4/495 of PPAs (0.8%). While addressing population-substructure through principal component analysis (PCA), we defined 2 patients groups with Central/Northern (n=873) and Southern European (n=523) ancestry.

The proportion of expansion carriers was significantly higher in bvFTDs compared to PPAs (5% vs. 0.8% [$p=2.17x10^{-5}$; OR=6.4; CI:2.31-24.99]), as well as in individuals with Central/Northern European compared to Southern European ancestry (4.4% vs. 1.8% [$p=1.1x10^{-2}$; OR=2.5; CI:1.17-5.99]). Pathogenic expansions and Central/Northern European ancestry independently and inversely correlated with

AAO. Our prediction model (based on expansions status, genetic ancestry and AAO) predicted a diagnosis of bvFTD with 64% accuracy.

Conclusions Our results indicate correlation between pathogenic *C9orf72* expansions, AAO, PCA-based Central/Northern European ancestry and a diagnosis of bvFTD, implying to complex genetic risk-architectures differently underpinning the behavioural and language variant syndromes.

Introduction

Frontotemporal lobar degeneration (FTLD) refers to the second most common form of young-onset dementia after Alzheimer's Disease (AD) [1]. The major clinical syndromes are behavioural variant (bvFTD) [2] and/or language dysfunctions, broadly called primary progressive aphasia (PPA); the latter is subdivided in semantic dementia (SD) (or semantic variant PPA) and progressive non-fluent aphasia (PNFA) (or nonfluent/agrammatic variant PPA) [2, 3]. FTLD can also occur together with motor neuron disease (FTLD-MND, or amyotrophic lateral sclerosis [FTLD-ALS]) in a continuous spectrum of phenotypes [4].

In FTLD, repeat expansions in *C9orf72* [5] have been previously reported to occur in ~25% [6-10] of familial and ~6% [11] of sporadic cases (i.e. individuals with no clear familial history and/or genetic aetiology [12]). Several studies had shown high frequencies of pathogenic *C9orf72* expansions in Northern vs. Southern European patients (North-South axis), especially in historically isolated populations (such as the Finnish [13, 14]), leading to the hypothesis that a Scandinavian founder might be at the basis of the spread of the *C9orf72* expansion [15]. Other studies (based on the geographical location of the recruiting sites) challenged the North-South axis concept either reporting a high frequency (~25%) of pathogenic expansions in the Spanish population [10], or implying to the existence of more than one risk-haplotype [16-19].

FTLD patients with abnormal *C9orf72* repeat expansions exhibit marked phenotypical and pathological heterogeneity, thus suggesting presence of additional (genetic and environmental) modifiers [20]. Despite conflicting studies reporting either direct or inverse correlation between repeat length and age at onset (AAO), *C9orf72* expansions have been suggested to act as a genetic modifier of AAO [16, 21-24].

We here analysed 1396 FTLD cases gathered through the IFGC (International FTD-Genetics Consortium; https://ifgcsite.wordpress.com/) phase-III initiative, aiming at (i) characterising *C9orf72* expansions in relation to genetic ancestry and AAO, and (ii) assessing the usefulness of these parameters in discriminating the behavioural from the language variant syndrome.

Methods

Cohort, clinical phenotyping

FTLD cases were collected between 2016 and 2018 (within the IFGC phase-III project [https://ifgcsite.wordpress.com/ongoing-projects/]). The samples were recruited by clinicians and research groups who are part of the IFGC network and based in Italy, Spain, Germany, the Netherlands, Belgium, UK, Sweden, Norway, Slovenia, and USA (**Supplementary Table 1:** https://doi.org/10.5522/04/12418157). Patients were diagnosed at each contributing site (**Supplementary Table 2:** https://doi.org/10.5522/04/12418157) in a harmonised fashion according to international consensus criteria such as the Neary *et al* (for FTLD), Rascovsky *et al* (for bvFTD), Gorno-Tempini *et al* (for PPA [SD or PNFA]) and Strong *et al* (for FTLD-MND) criteria [2-4, 25].

Genotyping, *C9orf72* repeat expansions and analysis cohorts

Thousand four-hundred and fifty-four (1454) cases were successfully genotyped by means of the NeuroArray [26] on the Illumina Infinium platform. Genotypes were used to inform on population substructure *via* standard principal component analysis (PCA) (**Supplementary Figure 1:** https://doi.org/10.5522/04/12418157), which led to the exclusion of 44 population outliers, and allowed to address population-

substructure within the cohort (we identified 2 distinct ['Nordic' and 'Mediterranean'] clusters; **Supplementary Figure 2:** https://doi.org/10.5522/04/12418157). We also assessed cryptic relatedness and excluded 14 first or second degree related individuals, leaving a cohort of 1396 cases (*group 0*) – for which *C9orf72* expansion status (i.e. presence/absence of pathogenic expansions) was known – for analyses. Frequencies of pathogenic expansions were assessed in *group 0* and further analyses were performed in: i) 1295 cases (*group 1*: n = 800 bvFTDs and n = 495 PPAs) with known *C9orf72* expansion status; ii) 1179 cases (*group 2*; n = 756 bvFTDs and n = 423 PPAs) with known *C9orf72* expansion status and age at onset (AAO) data available, and; iii) 734 cases (*group 3*; n = 462 bvFTDs and n = 272 PPAs) with AAO and repeat counts (rc; screened *via* repeat-primed PCR [RP-PCR] [c.f. [27, 28]], see **Supplementary Materials and Methods** and **Supplementary Figure 3:** https://doi.org/10.5522/04/12418157) data available (**Figure 1A**).

Statistical analyses

We first assessed the frequency of pathogenic expansions in the entire cohort (*group 0*). The information on presence/absence of expansions was used as a binary variable (0 = absence of expansion; 1 = presence of expansion). We then investigated differences in the frequencies of pathogenic expansions across bvFTDs and PPAs, and the 'Nordic' and 'Mediterranean' clusters in *group 1* (Fisher's Exact test) and in *group 3* (logistic regression); in the latter, we used repeat counts (rc) as a categorical variable (using 'no', 'short', 'intermediate' and 'long' as factor levels) considering the following 4 categories: 'no' expansions (rc = 2/3), 'short' expansions ($4 \leq rc \leq 8$), 'intermediate' expansions ($9 \leq rc \leq 24$) and 'long' expansions ($rc \geq 25$), the latter representing expansions in the pathogenic range (c.f. [10, 22]; see also

Supplementary Materials and **Methods** and **Supplementary Figure 3:** https://doi.org/10.5522/04/12418157).

We then evaluated association between AAO and syndrome, genetic ancestry and expansions (i.e. presence/absence used as a binary variable, see above) alone and with genetic ancestry as a covariate in *group 2* (t-test and logistic regression) and in *group 3* (t-test, ANOVA with Tukey *post-hoc* test, and logistic and linear mixed-effects model). In the latter case, we used rc as a categorical variable (see above).

Finally, we sought to build a model to predict syndrome (bvFTD vs. PPA) using (i) presence/absence of pathogenic expansions (as binary variable [see above] for *group 2*) or (ii) rc (as categorical variable [see above] for *group 3*), ancestry as binary variable and AAO as continuous variable using logistic regression models (i.e. the leave-one-out cross validation [LOOCV] and the K-fold models). A summary of the analyses workflow can be found in **Figure 1B**.

All analyses were performed using R studio (version 3.6.0, studio version 1.2.1335).

C9orf72 locus risk-haplotype

Twenty (rs1110264, rs1110155, rs2150336, rs1161680, rs2589054, rs1822723, rs4879515, rs895023, rs868856, rs1977661, rs903603, rs12349820, rs10122902, rs2282241, rs1948522, rs1982915, rs2453556, rs702231, rs696826 and rs247751) of the original 42 SNPs constituting the (Finnish) risk-haplotype [29] were available on the NeuroArray [26]. We filtered out 7 markers in order to keep 13 informative SNPs (rs1822723, rs4879515, rs868856, rs1977661, rs903603, rs10122902, rs2282241, rs1948522, rs1982915, rs2453556, rs702231, rs696826, rs2477518) matching 13 of the 20 used in Mok *et al* [15]. We evaluated the proportion of cases carrying at least one risk-allele (as in Mok *et al* [15]) for each marker assessing expansion vs. non-

expansion carriers (with/without ancestry stratification).

Standard Protocol Approvals, Registrations and Patient Consents

Each contributing site obtained written informed consent from all patients to be part of extended genetic studies; the current study is approved under Institutional Review Board (IRB),approval #9811/001.

Data availability

All data generated or analysed during this study are included in this published article (and **Supplementary files 1** and **2** at: https://doi.org/10.5522/04/12418157.

Results

C9orf72 expansions frequency and syndromes

We assessed the frequency of pathogenic expansions in the entire cohort and across the different syndromes in the *group 0* cases (**Figure 1**). Four percent of all cases (56/1396 [4%]) carried pathogenic expansions. These were most frequent in FTLD-MNDs (12/101 [11.9%]) followed by bvFTDs (40/800 [5%]) and PPAs (4/495 [0.8%]). The higher prevalence of pathogenic expansions in bvFTDs vs. PPAs was statistically significant (Fisher's Exact test: $p = 2.17x10^{-5}$; OR = 6.4; 95% CI: 2.31 – 24.99 **Table 1**). We further explored this finding in the *group 3* cases using logistic regression to assess association between expansion length (represented by 4 repeat counts [rc] factor levels – 'short', 'intermediate' and 'long' expansions, tested against 'no' expansions) and syndromes (bvFTD vs. PPA). Expansion length discriminated bvFTD from PPA with a trend that was significant in the 'intermediate' ($p = 4.7x10^{-2}$; OR = 1.6; CI: 0.0061 [2.5%] – 0.94 [97.5%]) and 'long' ($p = 1.9x10^{-3}$; OR = 7.2; CI:

0.86 [2.5%] – 3.45 [97.5%]) rc ranges (with a ~90% probability of a bvFTD diagnosis supported by the latter; **Supplementary Table 3** https://doi.org/10.5522/04/12418157).

C9orf72 expansions (and repeat counts [rc]) and genetic ancestry

We performed PCA (PC1 vs. PC2, **Supplementary Figure 2A**; PC1 vs. PC3, **Supplementary Figures 2B:** https://doi.org/10.5522/04/12418157) to cluster the *group 1* cases based on their genetic make-up. There were 2 major clusters: *cluster-1* ('Mediterranean') included most of the cases (439/500 [87.8%]) recruited from Southern European sites (Italy and Spain); *cluster-2* ('Nordic') included most of the cases (627/795 [78.8%]) recruited from Central and Northern European sites (Belgium, The Netherlands, Germany, UK, Norway and Sweden). Samples recruited from Eastern European (Slovenia) and North American sites distributed across both clusters – although with a higher prevalence within *cluster-2* (167/795 [21%]) vs. *cluster-1* (42/500 [8.4%]).

We observed a significantly higher prevalence of pathogenic expansions in the 'Nordic' (35/795 [4.4%]) vs. the 'Mediterranean' (9/500 [1.8%]) cluster (Fisher's Exact test: $p = 1.1x10^{-2}$; OR = 2.5; CI: 1.17 – 5.99 **Table 2**). We further evaluated this finding in the *group 3* cases using logistic regression to assess association between expansion length (see above) and genetic ancestry. Expansion length discriminated the 'Nordic' from 'Mediterranean' cluster with a trend that was significant in the 'intermediate' ($p = 9.7x10^{-4}$, OR = 2.2; CI: 0.32 [2.5%] – 1.25 [97.5%]) and 'long' ($p = 4.7x10^{-4}$, OR = 9.3; CI: 1.12 [2.5%] – 3.7 [97.5%]) rc ranges (with a ~90% probability of 'Nordic' ancestry supported by the latter; **Supplementary Table 4:** https://doi.org/10.5522/04/12418157).

Provided differences in syndromes prevalence and distribution across the 'Nordic' and 'Mediterranean' clusters – bvFTD (469/795 [59%] vs. 331/500 [66.2%]) and PPA (326/795 [41%] vs. 169/500 [33.8%]), respectively (**Supplementary Table 5:** https://doi.org/10.5522/04/12418157) – we analysed the distribution of pathogenic expansions across syndromes and clusters. Stratified Fisher's Exact test showed significant differences in the distribution of the pathogenic expansions between bvFTD and PPA in the' Nordic' (but not the 'Mediterranean') cluster ($p = 1x10^{-4}$; OR = 7.87; 95% CI: 2.43 – 40.52), and between the 'Nordic' and the 'Mediterranean' clusters for the bvFTD (but not PPA) syndrome ($p = 1.9x10^{-2}$; OR = 2.95; 95% CI: 1.31 – 7.52), suggesting that ancestry ('Nordic') and syndrome (bvFTD) are independently associated with pathogenic expansions (**Table 3**).

C9orf72 repeat expansions (and counts [rc]) and age at onset (AAO)

We assessed AAO in the *group 2* cases (**Figure 1**). Mean AAO was significantly different between the bvFTD (61.7) and PPA (64) syndromes (t-test: $p = 1.86x10^{-5}$; CI: -3.34 – -1.25), and the 'Nordic' (61.3) and 'Mediterranean' (64.3) clusters (t-test: $p = 1.16x10^{-7}$; CI: 1.86 – 4.03) (**Supplementary Table 6A** and **B**; **Figure 2A:** https://doi.org/10.5522/04/12418157). We then assessed the relationship between pathogenic expansions and AAO *via* logistic regression. First, we identified a significant correlation between a decrease in AAO and presence of pathogenic expansions ($p = 7.7x10^{-4}$; $R^2 = 0.008$; CI: -8.05 [2.5%] – -2.13 [97.5%]). When we included genetic ancestry in the model we observed a significant correlation with a decrease in AAO, no difference in using either cluster ($p = 2.3x10^{-3}$; CI: -7.5 [2.5%] – -1.63 [97.5%] for pathogenic expansions; $p = 2.3x10^{-7}$; CI: -3.9 [2.5%] – -1.77 [97.5%] for cluster; R^2=0.03) or PC1 ($p = 2.1x10^{-3}$; CI: -7.5 [2.5%] – -1.66 [97.5%] for pathogenic expansions; $p = 6.4x10^{-7}$; CI: 30.1 [2.5%] – 68.9 [97.5%] for PC1;

R^2=0.028) as covariate and an almost 4-fold goodness of fit increase (**Supplementary Table 7A**, **B** and **C:** https://doi.org/10.5522/04/12418157). Of note, when comparing the two regression models (with/without genetic ancestry as covariate) through the log-likelihood R^2 ratio test, the difference (between the 2 models) appeared not to be ~~not~~ due to chance ($p < 10^{-12}$) (**Supplementary Table 7B** and **C:** https://doi.org/10.5522/04/12418157).

We further evaluated the relationship between expansion length (represented by 4 repeat counts [rc] factor levels – 'short', 'intermediate' and 'long' expansions, tested against 'no' expansions) and AAO in the *group 3* cases (**Figure 1**). First, we independently analysed association between AAO and: i) genetic ancestry – mean AAO 60.9 and 64.6 in the 'Nordic' and 'Mediterranean' cluster, respectively (t-test: $p = 2.1x10^{-7}$; CI: 2.32 – 5.09; **Supplementary Table 8A:** https://doi.org/10.5522/04/12418157); ii) syndrome – mean AAO 61.7 and 63.5 in the bvFTD and PPA syndromes, respectively (t-test: $p = 9.1x10^{-3}$; CI: -3.11 – -0.44; **Supplementary Table 8B:** https://doi.org/10.5522/04/12418157), and; iii) expansion length – mean AAO 63.2 for both 'no' and 'short' expansions, 61 for 'intermediate' expansions and 58 for 'long' expansions (ANOVA: $p = 3.6x10^{-2}$; CI: -10.2 – -0.23 for 'long' vs. 'no' expansions) (**Supplementary Table 8D**; **Figure 2B** and **C:** https://doi.org/10.5522/04/12418157). We then assessed the relationship between expansion length (see above) and AAO *via* logistic regression. First, we identified a significant correlation between a decrease in AAO and both 'intermediate' and 'long' expansions ($p = 4x10^{-2}$; CI: -4.36 [2.5%] – -0.96 [97.5%] for 'intermediate' and $p = 7x10^{-3}$; CI: -9.05 [2.5%] – -1.43 [97.5%] for 'long' expansions; $R^2 = 0.017$) (**Supplementary Table 9A:** https://doi.org/10.5522/04/12418157). When we included genetic ancestry in the model we observed a significant correlation with a

decrease in AAO, no difference in using either cluster ($p = 4.7x10^{-2}$; CI: -7.65 [2.5%] – -0.05 [97.5%] for 'long' vs. 'no' expansion; $p = 2.38x10^{-6}$; CI: -4.73 [2.5%] – -1.97 [97.5%] for cluster; $R^2 = 0.045$) or PC1 ($p = 5.98x10^{-2}$; CI: -7.5 [2.5%] – 0.14 [97.5%] for 'long' vs. 'no' expansion; $p = 1.2x10^{-6}$; CI: 39.8 [2.5%] – 92.9 [97.5%] for PC1; R^2=0.047) as covariate and an almost 3-fold goodness of fit increase (**Supplementary Table 9A**, **B** and **C:** https://doi.org/10.5522/04/12418157). Of note, when comparing the two regression models (with/without genetic ancestry as covariate) through the log-likelihood R^2 ratio test, the difference (between the 2 models) appeared not to be due to chance ($p < 10^{-12}$) (**Supplementary Table 9B** and **C:** https://doi.org/10.5522/04/12418157). These findings were further supported by non-linear mixed-effects model regression using genetic ancestry as random effect covariate (for 'long' vs. 'no' expansion; see **Supplementary Table 10:** https://doi.org/10.5522/04/12418157).

C9orf72 locus risk-haplotype

All of the risk alleles for the 13 markers – shortest informative stretch of the original risk-haplotype [15, 29] available to us – were seen in: i) 40/56 (71.4%) expansion carriers vs. 380/1340 (28.4%) non-expansion carriers in the entire cohort; ii) 33/47 (70.2%) expansion carriers vs. 228/826 (27.6%) non-expansion carriers in the 'Nordic' cluster, and; iii) 7/9 (77.8%) expansion carriers vs. 152/514 (29.6%) non-expansion carriers in the 'Mediterranean' cluster. Comparing the proportion of risk-allele carriers (expansion vs. non-expansion carriers) for each single marker, 5/13 markers (rs4879515, rs868856, rs903603, rs2282241, rs2453556) were significant in the 'Nordic' cluster, none in the 'Mediterranean' cluster (**Supplementary Figure 4:** https://doi.org/10.5522/04/12418157). Rs2477518 showed variable frequencies for the risk-allele (T) across expansion vs. non-expansion carriers (and the 2 clusters),

thus making this most probably a negligible marker within this stretch, as hinted previously [15, 17]. Rs3849942, previously suggested as surrogate marker for the risk haplotype [15], was not among the SNPs available to us. We used rs868856, displaying strongest LD with rs3849942 (D'=0.96; R^2=0.7; https://ldlink.nci.nih.gov/), as informative proxy: the risk-allele segregated differently across expansion vs. non-expansion carriers in the 'Nordic' and 'Mediterranean' cluster (as for rs2453556) possibly suggesting these 2 as the most conserved markers of the original risk-haplotype across populations in expansion carriers (highlighted in blue in **Supplementary Figure 4:** https://doi.org/10.5522/04/12418157).

Syndrome prediction

We then sought to build a model to predict syndrome (bvFTD vs. PPA) and assess its accuracy. We analysed both *groups 2* and *3* cases using expansion status (presence/absence of expansion for *group 2*, and the 4 rc factor levels for *group 3* [see materials and methods]), genetic ancestry (using either 'cluster' or 'PC1') as binary variables, and AAO as a continuous variable in logistic regression models. We observed an accuracy of ~0.64 (*group 2*; **Supplementary Table 11:** https://doi.org/10.5522/04/12418157) and ~0.62 (*group 3*; **Supplementary Table 12:** https://doi.org/10.5522/04/12418157) in predicting bvFTD, whilst there were no differences in the outcome when using either 'cluster' or 'PC1' as covariates in both (LOOCV and K-fold) models.

Discussion

This study aimed to characterise *C9orf72* expansions in relation to genetic ancestry and age at onset (AAO), and to assess the usefulness of these parameters in discriminating the behavioural from the language variant syndrome, in a large pan-European cohort of 1396 FTLD cases.

To the best of our knowledge, the current work is unique in that, prior characterising the expansions, we excluded population-substructure bias using genome-wide genotyping data to cluster the cases on the basis of their genetic make-up. After principal component analysis (PCA) we identified two distinct clusters including samples with geographical ancestry corresponding to Southern Europe ('Mediterranean' cluster) and Central/Northern Europe ('Nordic' cluster). Our analyses not only showed that patients from the 'Nordic' cluster presented significantly higher frequency of pathogenic *C9orf72* expansions compared to the 'Mediterranean' cluster, but also that a core stretch of markers (n = 8) of the Finnish risk haplotype [29] appeared to be conserved across the 'Nordic' expansion carriers, whereas there was a similar tendency for (just) 2 of such markers in the 'Mediterranean' expansion carriers. Several studies had shown high frequencies of long *C9orf72* expansions in Northern vs. Southern European patients (North-South axis)[13-15]. Other studies (based on the geographical location of the recruiting sites) challenged the North-South axis concept [10], or the founder effect implying to the existence of more than one risk-haplotype [16-19]. All this taken together, our current data appear to support the North-South axis hypothesis and suggest that rearrangements (and instability) [16, 19] at the *C9orf72* locus might have occurred

reducing the level of conservation of the original risk haplotype across the European population.

We found pathogenic expansions in ~4% of all cases and that the proportion of expansion carriers was significantly higher in bvFTDs compared to PPAs. The fact that we overall identified significant association between pathogenic expansions and a diagnosis of bvFTD, and Central/Northern European ancestry – findings for the most in line with previous reports [8, 10, 13, 20, 30-34] – suggests that *C9orf72* expansions might serve as useful genetic fingerprint to define subpopulations of FTLD patients (**Figure 3**). Of note, we observed a trend of association with syndrome (bvFTD) and genetic ancestry (Central/Northern European) already supported by the 'intermediate' repeat counts ($9 \leq rc \leq 24$) category. This appears in line with previous reports suggesting that individuals with 7 to 24 alleles might have an increased risk to convert to carriers of pathological repeat expansions[10, 22] and may, altogether, be useful information in the context of diagnostics.

Despite some previous conflicting reports of direct (or inverse) correlation between *C9orf72* expansions and AAO [16, 21, 23], we (as others [22, 24]) found a significant inverse correlation between *C9orf72* expansion length and AAO. Additionally, and interestingly, our data also indicates that Central/Northern European genetic ancestry contributes to a decreased AAO (independently from the expansions) possibly implying to a more complex genetic signature (or architecture), and subsequently molecular mechanisms, underpinning this very feature. Clearly, disease mechanisms that involve *C9orf72* expansion length and AAO are complex, thus it is likely that additional factors might further modulate their relationship and effect on the phenotype (see also Babić Leko *et al* [5]).

While using expansion length, genetic ancestry and AAO in a regression model to discriminate behavioural from language variant subtypes, we found that such parameters did support a prediction of bvFTD with 64% accuracy.

Our results have a number of implications. First, provided that significant variation exists in the genetic architecture of the Caucasian population [35], genetic variability characterising and differentiating 'Nordic' vs. 'Mediterranean' subjects (such as in the case of our cohort) might influence predisposition to harbouring longer repeat expansions. In other repeat expansion diseases – e.g. Huntington's disease (HD) or other microsatellite diseases, including myotonic dystrophy and spinocerebellar ataxias [35] – the presence of specific haplogroups in Western European populations occurs with a manifold increase in prevalence of repeats compared to other ethnic groups and populations [36]. Second, different genetic risk-architectures underpinning different (and possibly genetically more homogeneous) subpopulations of patients may exist within the FTLD population.

In a nutshell, our results imply that a significantly higher proportion of FTLD cases, with 'Nordic' rather than 'Mediterranean' genetic ancestry, is likely to develop bvFTD in presence of 'intermediate' and 'long' (pathogenic) expansions, whilst 'long' (pathogenic) expansions are (almost) negligible in PPAs, regardless of ancestry. Clearly, multiple factors including genetic heterogeneity, epigenetic changes, ethnicity, as well as environmental factors and habits that may subsist within and across multicultural cohorts, all together, contribute to disease predisposition, onset and progression [22, 37, 38]. These concepts, reinforced by our study, warrant further characterisation of genetic, environmental, and additional clinical measures to fine-tune models able to predict disease outcome to complement diagnostic criteria, and

possibly assist, in the near future, in the identification of informative cohorts for tailored clinical trials and the development of effective personalised therapies.

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Figures legends

Figure 1. Cohorts (A) and analysis workflow (B).

AAO = age at onset; logistic/cluster = logistic regression using cluster as covariate; logistic/PC1 = logistic regression using PC1 as covariate; Loocv = leave one out cross validation regression model; k-fold regression model.

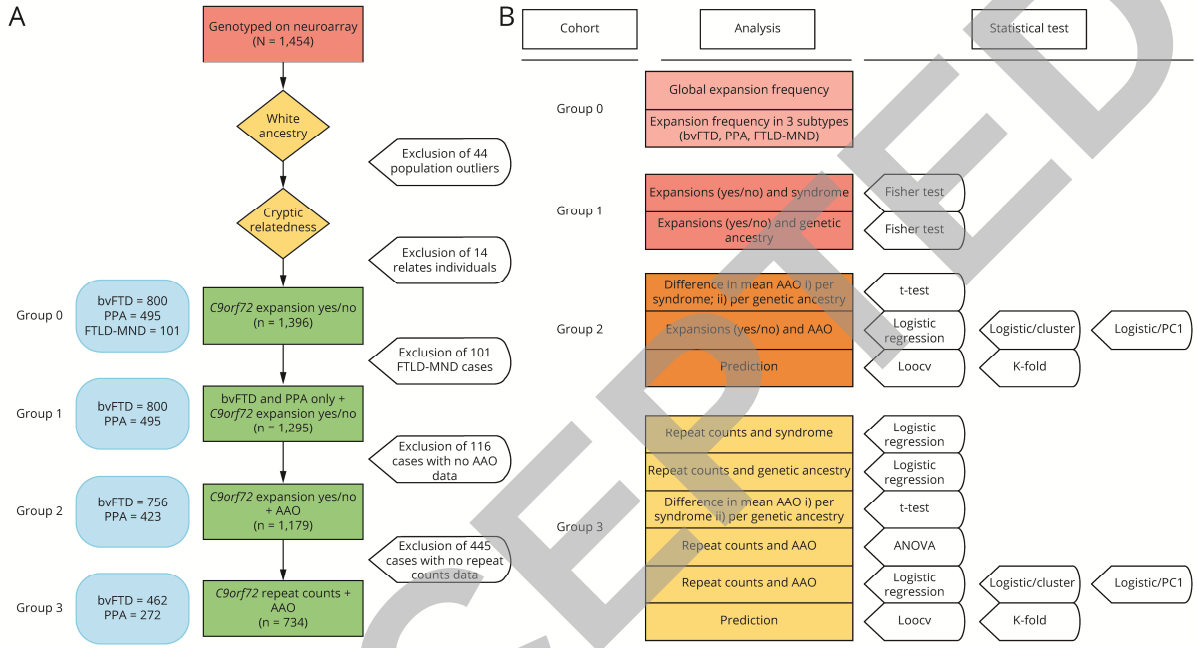


Figure 2. Association between AAO and: ancestry; syndrome; expansion length.

(**A**): AAO in the *group 2* cases. Mean AAO bvFTD (61.7) and PPA (64) (t-test: p = $1.86x10^{-5}$; CI: -3.34--1.25); mean AAO 'Nordic' (61.3) and 'Mediterranean' (64.3) clusters (t-test: p = $1.16x10^{-7}$; CI: 1.86-4.03). (**B**): AAO in the *group 3* cases. Mean AAO bvFTD (61.7) and PPA (63.5) (t-test: p = $9.1x10^{-3}$; CI: -3.11--0.44), mean AAO 'Nordic' (60.9) and 'Mediterranean' (64.6) (t-test: p = $2.1x10^{-7}$; CI: 2.32-5.09). (**C**): AAO in the *group 3* cases. Mean AAO for both 'no' and 'short' expansions (63.2), for 'intermediate' expansions (61) and for 'long' expansions (58) evaluated *via* ANOVA test.

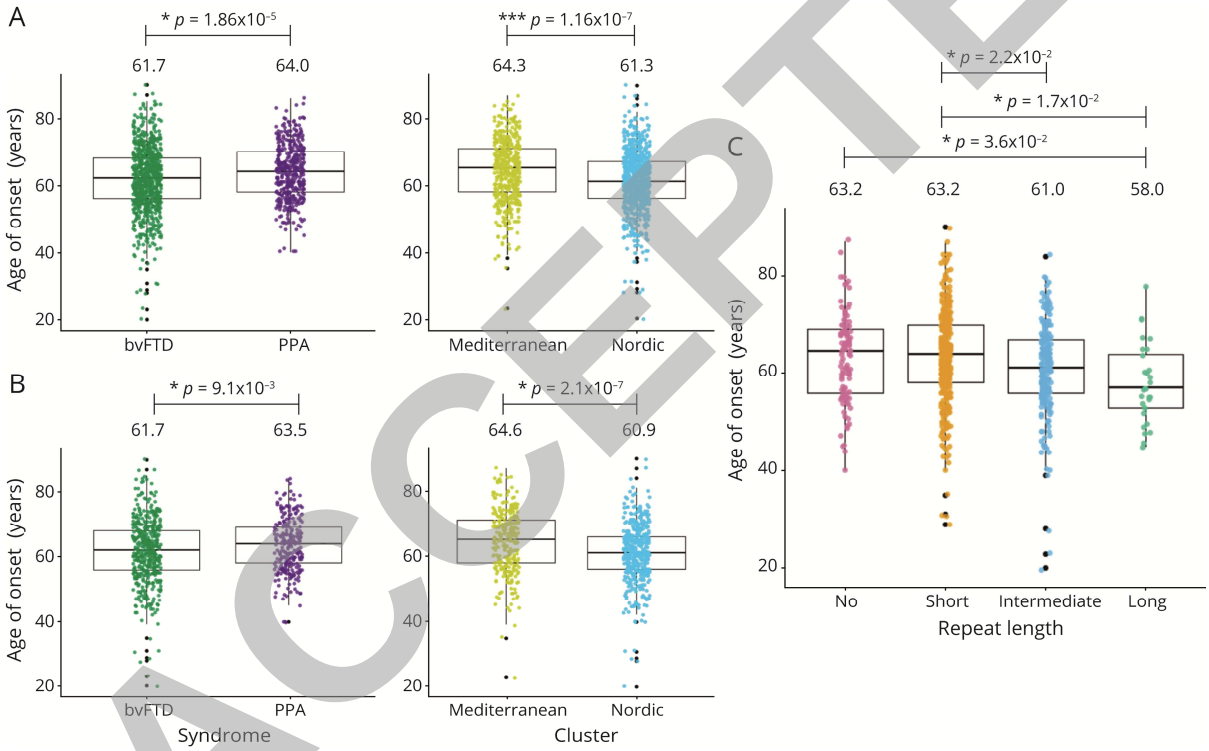
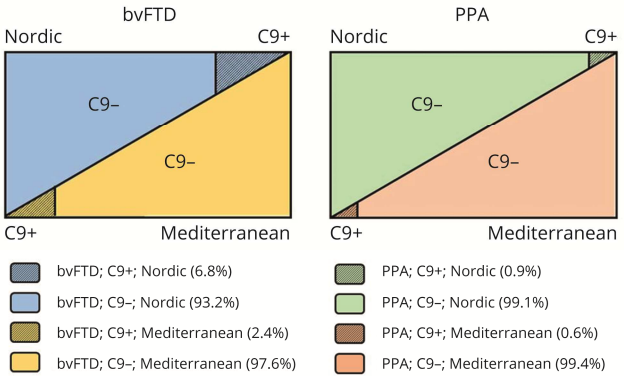


Figure 3. Patients subpopulations (bvFTD and PPA syndromes) based on C9orf72 expansions genetic signatures and ancestry.



Tables

Table 1. Frequency of expansion carriers in the entire cohort and by syndrome.

Summary of expansions carriers frequency in the entire cohort (n = 1396) and across syndromes. The higher prevalence of expansion carriers in bvFTD vs. the PPA is statistically significant: *Fisher's exact test performed to statistically evaluate the difference between the occurrence of pathogenic-expansions in the bvFTD vs. the PPA syndromes: $p = 2.17x10^{-5}$; odds ratio (OR) = 6.4; 95% confidence interval (CI): 2.31-24.99.

Cohort	n of cases	Expansion carriers	Frequency
bvFTD	800	40	5%*
PPA	495	4	0.8%*
FTLD-MND	101	12	11.9%
Total	1396	56	4%

Table 2. Frequency of expansion carriers in the 'Nordic' and 'Mediterranean' clusters.

The higher prevalence of expansion carriers in the 'Nordic' vs. the 'Mediterranean' cluster is statistically significant: *Fisher's exact test: $p = 1.1x10^{-2}$; OR = 2.5; 95% CI: 1.17-5.99.

Genetic ancestry	n of cases	Expansion carriers	Frequency
Nordic	795	35	4.4%*
Mediterranean	500	9	1.8%*

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tratified Fisher's exact tests comparing prevalence of pathogenic ıs across bvFTD and PPA and the 'Nordic' and 'Mediterranean' clusters.

P-values presented in the table are corrected for multiple testing statistics. Prior correction p-values were as follows: * (uncorrected) Fisher's exact test: $p = 4.7x10^{-3}$; OR = 2.95; 95% CI: 1.31-7.52 → significant difference in the prevalence of bvFTD expansion carriers in the 'Nordic' vs. the 'Mediterranean' cluster; # (uncorrected) Fisher's exact test $p = 2.7x10^{-5}$; OR = 7.87; 95% CI: 2.43-40.52 → significant difference in the prevalence of expansion carriers in bvFTDs vs. PPAs within the Nordic cluster.

Subtype/Ancestry	**Expansion range**		**Fisher's Exact Test**
bvFTD	**pathogenic**	**non-pathogenic**	
Mediterranean	8	323	$p = 1.9x10^{-2*}$
Nordic	32	437	

Ancestry/Subtype	**Expansion range**		**Fisher's Exact Test**
Mediterranean	**pathogenic**	**non-pathogenic**	
bvFTD	8	323	p = 1
PPA	1	168	
Nordic			
bvFTD	32	437	$p = 1x10^{-4\#}$
PPA	3	323	

C9orf72, AAO and ancestry help discriminating behavioural from language variants in FTLD cohorts

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